

Growth kinetics of *Salmonella* spp. pre- and post-thermal treatment[☆]

Vijay K. Juneja^{a,*}, Harry M. Marks^b

^a Microbial Food Safety Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA

^b U.S. Department of Agriculture, Food Safety Inspection Service, 14th and Independence S.W., Washington, DC 20250, USA

Received 21 January 2005; received in revised form 4 January 2006; accepted 5 January 2006

Abstract

This paper reports estimated growth kinetic parameters for a cocktail of stationary phase *Salmonella* serotypes, pre- and post-thermal inactivation treatment. Cells were grown in brain–heart infusion broth at 25 or 37 °C and then destruction of the cells was quantified at 55 °C using a submerged coil heating apparatus. The surviving cells (about 1–2 log₁₀ cfu/ml) were subsequently grown at 25 or 37 °C. The results indicated that lag phase duration times for the post-heat treated cells increased at 25 and 37 °C by about 6.2 h and at least 3 to 4 h, respectively, and thus the increases appear to be truly different. However, when considering the ratios of the lag phase duration times for post-treated to pre-treated cells, a significant difference was not found, where estimated ratios could exceed 4. Estimated exponential growth rates, EGR, were not affected by the treatment, where for 37 °C, EGR was estimated to be 0.9 log₁₀ (cfu/ml)/h, and at 25 °C, the EGR was estimated at 0.45 log₁₀ (cfu/ml)/h.

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Keywords: *Salmonella*; Lag phase; Exponential growth rates

1. Introduction

Salmonella is one of the most important foodborne pathogens of public health significance and continues to be a major concern to regulatory agencies and the food industry. The pathogen is a leading cause of gastroenteritis and a variety of foods including meat and poultry, milk, ice cream, cheese, eggs and egg products, chocolate and spices, have been implicated as vehicles of transmission (D'Aoust, 1989) for causing salmonellosis. In an effort to eliminate or guard against this pathogen, the United States Department of Agriculture (USDA) requires a 6.5-log₁₀ relative reduction of *Salmonella* spp., or that the probability of survival of any given *Salmonella* cell should be no more than 10^{−6.5}, for cooked beef, ready-to-eat roast beef and cooked corned beef products, and a 7-log₁₀ relative reduction in certain fully and partially cooked poultry products (USDA, 1999).

Microbial Risk Assessments (MRA) are used by US regulatory agencies (USDA-Food Safety Inspection Service)

in establishing performance standards or guidelines that, if followed, are assumed to provide a safe product. The MRA organizes information to describe or depict, clearly, the pathways through which a microbial hazard reaches its host. Typically, following the structure developed for chemical risk assessments, MRA consists of four compartments: hazard identification, exposure assessment, dose-response assessment, and risk characterization. The last step is an integration of the other three steps and basically provides estimates of the likelihood of illness from some hazardous agent (for example, *Salmonella*) faced by consumers of the product being considered. An important step in the exposure assessment analysis is the determination of the likelihood of cells surviving a lethality treatment and then (re)growing to high levels that might cause illness, if consumed. To model this possibility, it is necessary to have information concerning the growth kinetics of injured cells that were subject to some type of treatment meant to inactivate the cells.

This paper presents results of experiments that compare the growth kinetics of *Salmonella* spp. before and after being subjected to a thermal-lethality treatment. Specifically, the paper compares the growth characteristics — time spent in a lag phase, before entering an exponential phase of growth, and the exponential growth rate, of a cocktail of *Salmonella*

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* Corresponding author. Tel.: +1 215 233 6500; fax: +1 215 233 6697.

E-mail address: vjuneja@errc.ars.usda.gov (V.K. Juneja).

serotypes before lethality treatment to the growth characteristics of cells surviving the lethality treatment. We are not aware of studies with meat and poultry related pathogens, such as *Salmonella*, that have compared the growth kinetics of cells before and after an intervention treatment. In a study by Smelt et al. (2002), when lag times of individual cells of *Lactobacillus plantarum* were determined, both means and standard deviations of lag times increased for heat stressed injured cells. It seems reasonable to assume that surviving or viable cells of a population of cells that were subjected to a lethality treatment would be injured, and if the surviving cells were placed in an environment where they could grow, the lag phase duration for the cells before entering an exponential phase of growth would be greater than it would be if the cells were not injured because the lag time for the injured cells would include time for cell recovery due to injury, as well as an adjustment period that explains the lag phase duration for uninjured cells. The different degrees of injury and ability to recover among cells, subjected to the same treatment, from the same population, would cause an increased variability of lag phase times for injured cells compared to uninjured cells that were in a stationary phase, as observed by Smelt et al. (2002).

Growth temperatures of cells, after injury, may affect their rate of recovery from injury; for example, a higher temperature could speed the recovery time from injury compared to that at a lower temperature. The cell mechanisms causing cell recovery from injury and acclimation to an environment may be occurring, at least partially, in parallel, both being affected by temperature and time. Over widely different temperatures, the relationship between expected lag times for pre- and post-thermal treated cells might not be described simply, for example, by a single factor or difference, or a simple linear regression. This research represents an initial exploration of the relationship.

Regarding the exponential growth rates, the expectation would be that there would be no discernable effect of the lethality treatment on exponential growth rates since these are generally not thought of as being dependent upon previous history: once cells grow and divide, the offspring cells would be in the exponential phase and the relative rates of growth would be the same, and not a function of the status or history of the offspring's progenitors. However, for example, it might be that surviving cells after a potentially lethal treatment, are endowed with genes that enhance, or inhibit growth, as well as survivability, compared to typical cells of the populations – the ones more likely to succumb to the treatment – and thus, the offspring of surviving cells would grow at faster (or lower) rates than typical cells from the original populations.

The lethality treatment designed for this study is considered to be severe at 55°C, and achieved reductions of 3–6 log₁₀ at 37 °C, and of 6 to 8 log₁₀ at 25 °C. The two temperatures studied in this research represent a region where the growth of *Salmonella* is rapid, and thus is of particular interest if the product was abused at these temperatures since the lag-phase duration times for the uninjured cells could be small.

2. Materials and methods

2.1. Bacterial serotypes

Salmonella Typhimurium (strain designation H3380), a human clinical isolate, originally obtained from CDC was used in this study. This serotype was stored at –70 °C in a mixture (85 : 15; v/v) of Tryptic Soy Broth (TSB; Difco; Detroit, MI) and glycerol (Sigma Chemical Co., St. Louis, MO).

2.2. Preparation of test cultures

To prepare the cultures, vials were partially thawed at room temperature and 1.0 ml of the culture was transferred to 10 ml of brain heart infusion broth (BHI; Difco) in 50-ml tubes and incubated for 24 h at 37 °C. This culture was not used in growth studies as it contained freeze-damaged cells. A working culture for use in growth and heating studies was prepared by transferring 0.1 ml of each culture to 10 ml tubes of BHI and incubating aerobically for 24 h at 37 °C. These cultures were maintained in BHI for 2 weeks at 4 °C. A new series of cultures was initiated from the frozen stock on a biweekly basis.

A day before the experiment, the inocula for conducting the growth and heating studies were prepared by transferring 0.1 ml of each culture to 10 ml tubes of BHI, and incubating aerobically for 18 h at 37 °C to provide late stationary phase cells. On the day of the experiment, each culture was centrifuged (5000 ×g, 15 min, 4 °C), the pellet was washed twice in 0.1% peptone water (wt/vol) and finally suspended in peptone water to a target level of 8–9 log₁₀ cfu/ml. The population densities in each cell suspension were enumerated by spiral plating (Model D; Spiral Biotech, Bethesda, MD) appropriate dilutions (in 0.1% peptone water), in duplicate, on Tryptic soy agar (TSA; Difco) plate and incubating at 37 °C for 48 h.

2.3. Growth experiments

Brain heart infusion broth (BHIB, 100 ml) in 250 ml flasks was sterilized for 15 min at 121 °C. Each flask was inoculated with 0.1 ml of the diluted inoculum of an 18-h culture of *Salmonella* to yield a starting level of approximately 2–3 log₁₀ cfu/ml, and then incubated at 25 or 37 °C on a model G-26 rotary shaker (120 rpm). At intervals appropriate for the temperature, samples were withdrawn for determining the bacterial number by serial dilutions in peptone water, surface plating with a spiral plater onto TSA as described above. Counts for two 0.1 ml samples were made.

2.4. Thermal inactivation procedure

Bacterial suspensions obtained were heated at 55 °C using a submerged coil heating apparatus (Cole and Jones, 1990). The submerged coil heating apparatus is comprised of a stainless steel coil fully submerged in a thermostatically controlled water bath which allows microbial suspensions to be heated between 20 and 90 °C with a short time to achieve temperature equilibrium. During the heating procedure, samples (0.2 ml) were

removed at predetermined time intervals. Where low cell numbers were expected, 0.6 ml aliquots were removed. Samples were cooled rapidly in ice slurry.

2.5. Enumeration of surviving bacteria

Decimal serial dilutions were prepared from duplicate samples at each sampling time in peptone water and appropriate dilutions were surface plated, in duplicate, on TSA, supplemented with 0.6% yeast extract and 1% sodium pyruvate, using a spiral plater. Samples not inoculated with *Salmonella* were plated as controls. Also, 0.1 and 1.0 ml of undiluted suspension were surface plated, where relevant. All plates were incubated at 30 °C for at least 48 h prior to counting colonies. For each sample, the counts obtained from the 2 plates were averaged and the average was then multiplied by the appropriate factor to estimate the level (cfu/ml) of *Salmonella* cells.

2.6. Re-growth experiment

Samples subjected to heating for the duration of the thermal inactivation experiments were quickly cooled. Heated suspensions (0.2 ml) was inoculated in BHIB (100 ml) in 250 ml flasks and then incubated at 25 or 37 °C on a model G-26 rotary shaker (120 rpm). Population densities were determined as above.

2.7. Study design

For growth at 37 °C, 18 replicate experiments (growth and regrowth after lethality treatment) were conducted in clusters over 6 different periods (three each period). For growth at 25 °C, 8 replicates were performed (growth and regrowth at 25 °C) in two periods. For the first period, 2 replicates were performed; for the second period, 6 replicates were performed. Replicates within each period of time were performed independently.

2.8. Statistical methods

Growth curves for each pre- and post treatment experiment were estimated assuming, a two-compartmental growth model, where cells are either in a lag phase or an exponential phase (Juneja et al., 2003):

$$\ln(\hat{N}(t)) = n_0 + \ln \left[\frac{\lambda e^{\mu t} + \mu e^{-\lambda t}}{\lambda + \mu} \right] + \varepsilon \quad (1)$$

where $\hat{N}(t)$ is the estimated level of *Salmonella* at time t (cfu/ml), n_0 , μ , and λ are parameter values to be estimated, ε is a residual error term at time t , assumed to be normally distributed with expected value equal to 0 and standard deviation equal to σ . The parameter μ characterizes the exponential growth rate, defined to be proportional to the instantaneous conditional probability, or hazard, of a cell in the exponential phase to divide. The parameter λ in effect characterizes the lag time, defined to be proportional to the instantaneous conditional probability that a cell in the stationary phase would “switch” to the exponential phase (Baranyi, 1998). It is assumed that these parameters are

not functions of time, t . The lag time, LAG, is the time delay for a population of cells to exhibit exponential growth, and is defined, geometrically, to be the intersection of the asymptotic line of Eq. (1), assuming $\varepsilon=0$, and horizontal line at n_0 . In terms of the parameters of Eq. (1),

$$\text{LAG} = \mu^{-1} \ln(1 + \mu/\lambda). \quad (2)$$

Of interest is the relationship between the LAG times as a function of the treatments (pre- and post-lethality) and temperature. Thus, differences and ratios were examined. When examining ratios, actually the logarithm of the LAG was used, so that ‘average’ ratios were computed by considering differences of logarithms, which are transformed by exponentiation, to compute the geometric mean. To determine interaction effects of temperature and treatment, a difference of differences was examined, or, equivalently, interactions terms within an analysis of variance.

The exponential growth rate, EGR, customarily is expressed in \log_{10} units, so that EGR measures the rate of increase, on a per hour basis, of logarithm base 10 of the *Salmonella* level (noted here in units as \log_{10}/hour). Thus,

$$\text{EGR} = \mu/\ln(10). \quad (3)$$

Eq. (1) represents a growth curve for which there would be expected no initial decline of cells. In other words, it is expected that all cells initially in the lag phase would eventually reach an exponential phase of growth. However, this assumption may not be true, particularly if the cells for which growth is to be observed were injured due to some prior treatment (such as those surviving a heat treatment). Let $k(t)$ be the hazard function associated with the cells surviving the lethality treatment; that is $k(t)$ is the slope of the survival curve for a level of N_0 at $t=0$. However, some of these cells, assumed to be in some sort of lag phase where the cells are repairing themselves, convert to an exponential phase of growth, while others will be inactivated. The differential equations that describe the relationships of the levels over time of cells that are either still in lag phase, $N_0(t)$, and in exponential phase, $N_e(t)$ can be written as:

$$\begin{aligned} \frac{dN_0(t)}{dt} &= (k(t) + \lambda)N_0(t) \\ \frac{dN_e(t)}{dt} &= \lambda N_0(t) + \mu N_e(t) \end{aligned} \quad (4)$$

$$\text{Let } \gamma(t) = \int_0^t k(s)ds \text{ and } H(t) = \lambda \int_0^t e^{-[\gamma(s) + (\lambda + \mu)s]} ds.$$

The solution to Eq. (4) is:

$$\begin{aligned} N_0(t) &= N_0 e^{-(\gamma(t) + \lambda t)} \\ N_e(t) &= N_0(t) e^{\mu t} H(t) \end{aligned} \quad (5)$$

There is not enough data to estimate a complex function of $k(t)$. One simple assumption is that $k(t)$ is constant ($=k$). With

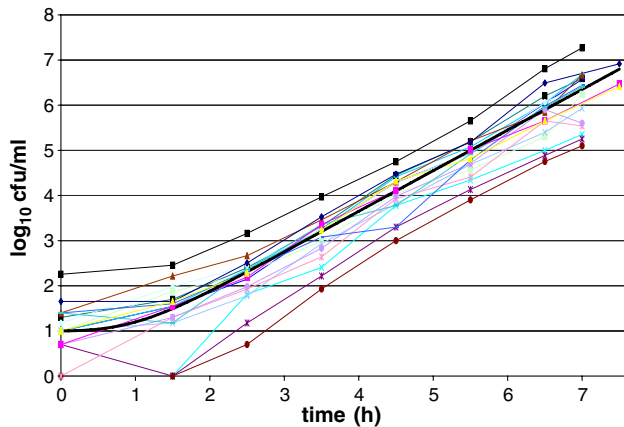


Fig. 1. Plots of observed growth curves: \log_{10} (cfu/ml) versus time, of *Salmonella* at 37 °C, before lethality treatment at 55 °C. Measured points are connected by straight lines. The smoothed dark line is a growth curve with parameters determined from a statistical fit of these data, as explained in the paper.

this assumption, the expected level of total cells: $\Psi(t) = N_0(t) + N_c(t)$ is:

$$\Psi(t) = \frac{N_0}{k + \lambda + \mu} \left(\lambda e^{\mu t} + (k + \mu) e^{-(k+\lambda)t} \right) \quad (6)$$

When $k=0$ Eq. (6) reduces to Eq. (1), ignoring the error term, where the expected value of $N^*(t)$ is $\Psi(t)$.

For 37 °C, the actual counts of the number of colony forming units on some of the plates were small (less than 10). Thus for this temperature, the actual estimates of the parameter values were made using Poisson regression: it was assumed that the sum of counted colony forming units on the two plates was distributed as a Poisson distribution, with expected value $f\Psi(t)$, where f is the appropriate factor, depending on the dilution, used to estimate the level of colony forming units per milliliter.

Nonlinear mixed effects models were used to estimate parameters of Eqs. (1) and (6). Calculations were performed on PC-SAS®, Release 9.0.

Table 1
Estimated exponential growth rates (EGR) and lag times of *Salmonella* at 37 °C by periods of experiment

Day	Lag		Egr	
	Mean	Std	Mean	Std
1	0.829	0.459	0.854	0.055
2	0.487	0.843	0.907	0.046
3	1.182	0.097	0.944	0.007
4	1.332	0.115	0.851	0.032
5	0.839	0.734	0.909	0.038
6	1.662	0.281	0.933	0.027
Mean	1.055	0.591	0.900	0.050

Each period consists of three growth experiments.

The standard deviations in the last row (mean) are based on an analysis of variance with day as a random factor: specifically it equals the square root of the sum of the between-day and within-day variance components, derived from the standard AVOVA. The standard errors of the means are: 0.171 for the mean lag; and 0.0159 for the mean EGR.

3. Results

3.1. Results at 37 °C

3.1.1. Pre-treated growth curves

Eighteen observed *Salmonella* growth curves at 37 °C, \log_{10} (cfu/ml) versus time, t (hours) were obtained for up to 7–7.5 h., with consecutive points connected by straight lines, for the pre-treatment data are depicted in Fig. 1. For ND samples, a value of 0 was assigned to the plotted \log_{10} level. The smoothed darkest lines are plots of the fitted Eq. (1) with parameter values derived from the average LAG and EGR values given in Table 1, assuming an initial level of 1 \log_{10} cfu/ml. The observed growth curves are similarly shaped, for the most part displaying an exponential growth following a lag period, suggesting that the cells did not reach a stationary phase during the experiment. However, two data points, at time equal to 7 h for which the measured levels were less than the measured levels at time equal to 6.5 h for the corresponding growth curves, were deleted.

The means of the estimated EGR values and LAG values for the pre-lethality treatment groups were 0.90 \log_{10} (cfu/ml)/h and 1.06 h, respectively, with standard errors of 0.016 and 0.17, respectively, computed from an analysis of variance, where period of time was considered a random factor. For EGR there was a significant day effect (P -values=0.03), while for the lag time there was not (P -value=0.15). Within day, the EGR and LAG estimates were correlated, with correlation coefficient of 0.4; however, the between-day correlation was close to zero (0.03).

3.1.2. Post-lethality treated growth curves

Growth was observed in 6 of the 18 experiments; for 3 of the experiments, there was observed declines of more than 2 \log_{10} cfu/ml before growth was observed for the other three experiments there were observed only a slight reduction of levels of *Salmonella*. Fig. 2 presents plots of the observed growth curves for the 6 experiments for which growth was observed. Fitted growth curves, based on Eq. (6) (assuming a constant

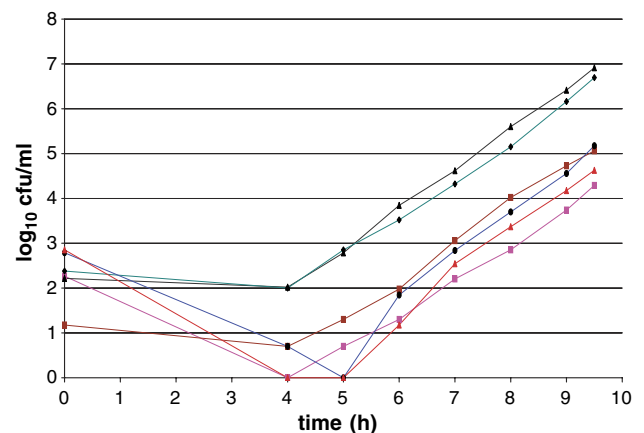


Fig. 2. Plots of observed growth curves: \log_{10} (cfu/ml) versus time, of *Salmonella* at 37 °C, after the lethality treatment at 55 °C. Measured points are connected by straight lines.

Table 2
Estimated exponential growth rates (EGR) and lag times of *Salmonella* at 25 °C by treatment periods (pre- and post-lethality) and time period of experiment

Period	EGR		Lag time	
	Pre	Post	Pre	Post
1	0.41	0.46	3.26	9.72
2	0.45	0.46	1.94	8.23
Mean	0.43	0.46	2.60	8.97

The first period consisted of 2 experiments; the second period consisted of 6 experiments. The average obtained lethality (\log_{10} relative reductions) obtained for the 2 periods were: 5.78 and 7.37, respectively.

inactivation rate, k) to the three experiments for which there were not observed a large reduction of levels of *Salmonella*. For these three curves, the mean EGR and LAG times (with standard errors in parentheses) were: 0.892 (0.0169) \log_{10} (cfu/ml)/h and 4.55 (0.132), respectively. For the other three curves, just examining the linear portions of the curve, using data for 6 h or more, the average of the estimated EGR values is 0.90, very close to the average estimated EGR obtained for the other three growth curves.

3.2. Results at 25 °C

Eight *Salmonella* growth curves at 25 °C were observed. Two results from the pre-treatment growth data were deleted from the analysis: 1) a reported result of 2.56 \log_{10} cfu/ml for one curve at time=0 min, which was higher than the other reported values at the same time, and was greater than the reported value of 1.85 at 2 min by 0.81 \log_{10} cfu/ml; and 2) a reported result of 4.7 \log_{10} cfu/ml for another curve at time=6.5 min, which was higher than the other reported values at the same time, and was greater than the reported value of 1.85 at 8.5 min by 0.30 \log_{10} cfu/ml. In graphs, not presented here, these points can be clearly as being deviant.

Table 2 presents estimated values of the lag times and EGR, by treatment group and period of time of the experiment. The means of the estimated EGR for the pre- and post-lethality treatment group were 0.43 and 0.46 (cfu/ml)/h, respectively, for an average of about 0.45 (cfu/ml)/h. The means of the period-specific estimated LAG for the pre- and post-lethality treatment group were 2.60 and 8.97 h, respectively, for a difference of 6.37 h.

4. Discussion

At 25 °C, there was no evidence of an initial decline in the cell-levels for the post-treated cells, as occurred for the cells for which growth took place at 37 °C. The difference could be explained by slight differences in experimental procedures that affected the cells differentially regarding their ability to re-grow after a lethality treatment. Reasons for the difference are unknown. However, from the curves for which growth was observed estimates of the relationship of the growth kinetics for pre- and post-treated cells can be derived.

Our prior expectation, as explained in the Introduction, was that, for injured surviving cells when re-grown at the same

temperatures as uninjured cells, lag times would increase and exponential growth rates would remain the same. The data analysis supports these conclusions. Analyses given below concern the difference in lag phase durations between the two treatments and the ratio of them.

At 37 °C, it appears that, when growth occurred, the estimated lag times increased at least by about 3–4 h, from about 1 h for pre-treated cells to more than 4 h for the post-treated cells. For the three growth curves for which there was a small decline of the levels of *Salmonella*, (referred to in this discussion as the 3₁ set of curves) the average of the estimated lag times, using Eq. (6), was close to 4.5 h. However, this estimate is based on an assumption that the actual decline in the cell-levels, before growth, was small, or, in other words, based on an assumption that between the two measurements at $t=0$ and $t=4$ h there was not a large decline followed by some growth. In other words, it is possible that for the 3₁ set of curves, cells entered the exponential phase before 4 h, proceeded by a greater decline in levels of *Salmonella*.

In comparison, for the three growth curves for which there were measured large declines (referred to as the 3₂ set of curves), it appeared (Fig. 2) that the exponential growth phase was entered after 4 h, since at 4 h two of the three curves had non-detectable levels, and the other curve had a non-detectable level at 5 h. For the 3₂ set of curves, both at 4 and 5 h, only one cell was found. That is, in 0.6 ml of sample (0.2 ml for each growth curve) for both 4 and 5 h, the number of cells found was 1. Finding one cell implies that the expected value of the underlying Poisson distribution is less than 6.638 cells, since if this were the expected value, the probability of seeing one or fewer cells is 1%. This expected number of cells in 0.6 ml (assuming, for the sake of argument, the underlying distribution to be the same for all three growth curves) implies an expected level of about 10 cells/ml, or in logarithmic units, 1 \log_{10} . In other words, the findings at both 4 and 5 h for these three curves imply that, ignoring the lack of 100% recovery, the level is very likely less than 1 \log_{10} cells/ml. If recovery were only 75%, then levels would be less than 1.17 \log_{10} . Given EGR of 0.9 \log_{10} (cfu/ml)/h, a level of 1.17 \log_{10} could be obtained in 1.3 h starting from 1 cell per ml (0 \log_{10}). Thus, for the 3₂ set of curves, while it is not possible to exclude a lag phase duration less than 4 h, it seems that it could not be much less than 4 h and is most likely greater than 4 h, possibly even greater than 4.5 or 5 h.

The observed results for both the 3₁ and 3₂ set of curves, consequently, could be explained consistently by assuming that the actual lag phase durations for the growth curves are similar in magnitude and would exceed 4 h. Accepting this explanation, from the 3₁ set of curves, the estimated lag phase duration would be the average of the three growth-curve specific estimates, 4.55 h, with a corresponding 90% confidence interval (using a t -distribution with two-degrees of freedom) of 4.16 to 4.94 h. Since the estimated lag phase duration for the pre-treated cells was 1.06 h, the ratio of the lag phase durations for post-treated cells to that of pre-treated cells is, 4.55/1.06=4.3, with a standard error of 0.72, computed using the relationship that the relative variance of a ratio is the sum of the relative variances of

the numerator and denominator when these are statistically independent. To determine a confidence interval for this ratio, it is assumed normal distributions for the numerator and denominator; the moments of the variance of the ratio are equated to that of a chi-square to derive the effective degrees of freedom associated with the standard error of the ratio; and a confidence interval is constructed using a *t*-distribution with the calculated effective degrees of freedom. This turns out to be nearly 15, from which a 90% confidence interval for the ratio (estimated to be 4.3) of the lag phase durations of the post-treated to pre-treated cells is 3.1 to 5.6.

A similar analysis for the difference in the lag phase durations is performed, with the results that the mean difference is 3.5 h, with a 90% confidence interval of 3.1 to 3.9 h.

At 25 °C, with respect to the ratio, a mixed effects model was performed, where the dependent variable was the estimated log of the lag phase duration for each growth curve, using treatment as a fixed factor and period as a random factor. From this analysis, an estimate of the ratio of the lag phase duration for the post-treated to pre-treated cells was derived to be equal to 4.1, with a 90% confidence interval of 2.8 to 5.4. This interval is nearly the same as the one computed above for 37 °C.

For the difference of the lag phase duration at 25 °C for the two treatment groups, using the mixed effects model described above with the estimated lag phase durations as the dependent variable, for the pre- and post-treated cells the mean estimated lag phase durations were estimated to be 8.5 and 2.3 h, respectively, resulting in an estimated treatment effect of 6.2 h, with 90% confidence interval of 4.5 to 7.7 h. This confidence interval does not overlap the corresponding confidence interval calculated for the difference of lag phase durations at 37 °C.

5. Conclusion

Using the results of the study of growth of cells at 25 and 37 °C pre- and post-thermal lethality treatment (at 55 °C), estimates of the exponential growth rates and lag-phase

durations for post-treated cells and the pre-treated cells were made. As expected, there was no significant treatment effect regarding the exponential growth rates: estimated exponential growth rates at 25 and 37 °C were about 0.45 and 0.90 (log₁₀ cfu/ml)/h, respectively. For growth at 37 °C the lag phase duration were estimated, on average, to be 1.1 h for pre-treated cells, and greater than 4 h and possibly more than 4.5 h for post-treated cells. Consequently, the ratio of the estimated lag phase duration of post-treated cells to pre-treated cells is likely to be more than a factor of 4. For growth at 25 °C, the ratio of the two lag phase durations was estimated to be about 4.1 with 90% confidence interval of 2.8 to 5.4. The lag phase durations at this temperature were estimated, on average, to be 2.3 h, pre-treated and 8.5 h post-treated, for an increase of about 6.2 h (with 90% confidence interval of 4.5 to 7.7 h). Thus it appears that, the difference of lag phase duration between post-treated and pre-treated cells is greater at 25 °C when compared to that at 37 °C; however, for the two temperatures, the ratios of the two lag phase durations (post-treated to pre-treated) cannot be said to differ significantly. Further study is planned to clarify this relationship as a function of temperature.

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